Tightly Controlled
Bacterial Protein Expression

The pBAD system offers:

- Tightly regulated expression
- Dose-dependent induction
- High protein yields
- Simplified detection and purification of expressed protein

Controlled expression for maximum yields

Take control of expression in *E. coli* with the pBAD Expression System. Tight regulation allows you to turn expression on or off. Dose-dependent induction lets you easily modulate expression levels. No other bacterial expression system gives you such control.

The pBAD Expression System helps you overcome two of the most common problems for heterologous protein expression in bacteria:

- Toxicity of the recombinant protein to the host
- Insolubility of the recombinant protein when it is expressed at high, uncontrolled levels

In both cases, a tightly regulated expression system is critical for maximizing recombinant protein yields. Based on the *araBAD* operon, which controls the arabinose metabolic pathway in *E. coli* (1,2,3), the pBAD Expression System allows you to precisely modulate levels to optimize yields.

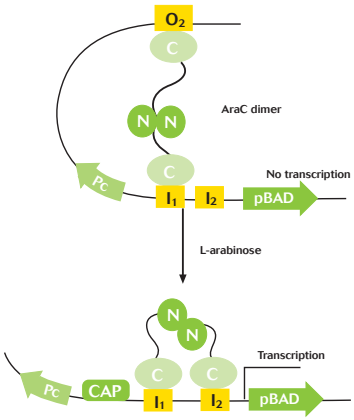
Tight regulation

The pBAD vectors' unique design give you precise control of expression levels. The *araBAD* promoter initiates gene expression. It's both positively and negatively regulated by the product of the *araC* gene (4), a transcriptional regulator that forms a complex with L-arabinose. In the absence of arabinose, the AraC dimer contacts the O_2 and I_1 half sites of the *araBAD* operon, forming a 210 bp DNA loop (Figure 1). For maximum transcriptional activation, two events are required:

- Arabinose binds to AraC. The protein releases the O_2 site and binds the I_2 site, which is adjacent to the I_1 site. This releases the DNA loop and allows transcription to begin (5).
- The cAMP activator protein (CAP)-cAMP complex binds to the DNA and stimulates binding of AraC to I_1 and I_2.

Basal expression levels can be repressed by introducing glucose to the growth medium. Glucose acts by lowering cAMP levels, which in turn decreases the binding of CAP. As cAMP levels are lowered, transcriptional activation is decreased. This is ideal when the protein of interest is extremely growth-inhibitive or toxic to the host (3).

Figure 1 - Regulation of the *araBAD* promoter

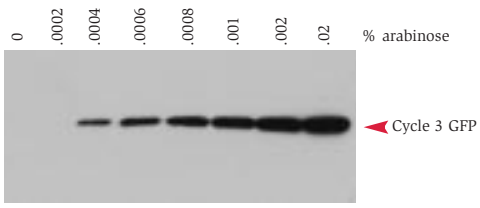


Precise control for optimal yields

With pBAD, you're in control throughout the entire cell growth and expression process. Easily optimize induction and identify the conditions that are best suited to the unique properties of your protein.

Figure 2 demonstrates the uniquely tight, arabinose-dependent induction of Cycle 3 GFP (green fluorescent protein) from pBAD/His. The data show the distinctive low levels of uninduced expression as well as the tight, dose-dependent induction of GFP in the presence of increasing concentrations of arabinose.

Figure 2 - Western blot of arabinose induction

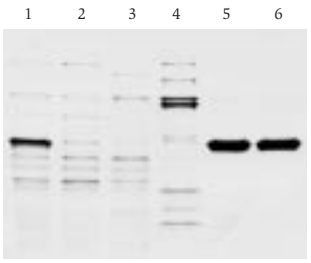


TOP10 bacterial cultures containing pBAD/His/GFP were grown to mid-log phase (A600 ~0.5 OD) and induced for 3 hours with increasing doses of arabinose. One milliliter of cell culture was centrifuged and the pellet resuspended in 100 µl of lysis buffer. Ten microliters of cell lysate was separated by SDS-PAGE and blotted. Protein was detected using the Anti-Xpress™ Antibody and chemiluminescence.

Simplified purification

Detecting and purifying proteins expressed from the pBAD vectors is simplified by the presence of epitope tags. Figure 3 demonstrates the simple process of expression and purification of the 53 kD Positope™ Control Protein*. With pBAD, it's easy to achieve maximized expression, specific protein detection and efficient purification.

Figure 3 - Purification of the Positope™ Control Protein



The Positope™ Control Protein was expressed in TOP10 *E. coli* from the pBAD/Thio-TOPO® vector and purified using ProBond™ Resin. Four micrograms of total protein was loaded into each lane of an SDS-PAGE gel.

Lane 1: Crude extract
Lane 2: Flow through
Lane 3: Column wash
Lane 4: 100 mM imidazole elution
Lane 5: 500 mM imidazole elution
Lane 6: Purified, dialyzed Positope™ Protein

* The Positope™ Control Protein is a recombinant protein specifically engineered to contain seven different tags for detection with seven different antibodies available from Invitrogen. The Positope™ Control Protein is intended for use as a positive control for antibody function in western blot experiments.

Variety and versatility

Invitrogen's pBAD vectors are specifically designed for maximum expression and ease of use. Nine pBAD vectors are currently available: pBAD102/D-TOPO®, pBAD202/D-TOPO®, pBAD-TOPO®, pBAD/Thio-TOPO®, pBAD/His, pBAD/*Myc*-His, pBAD-DEST49, pBAD/gIII and pBAD/Thio-E.

A variety of vector-specific features are available to facilitate cloning, protein purification and detection. With so many pBAD vectors to choose from, you're sure to find one that fits your needs.

The following features are included in all pBAD vectors:

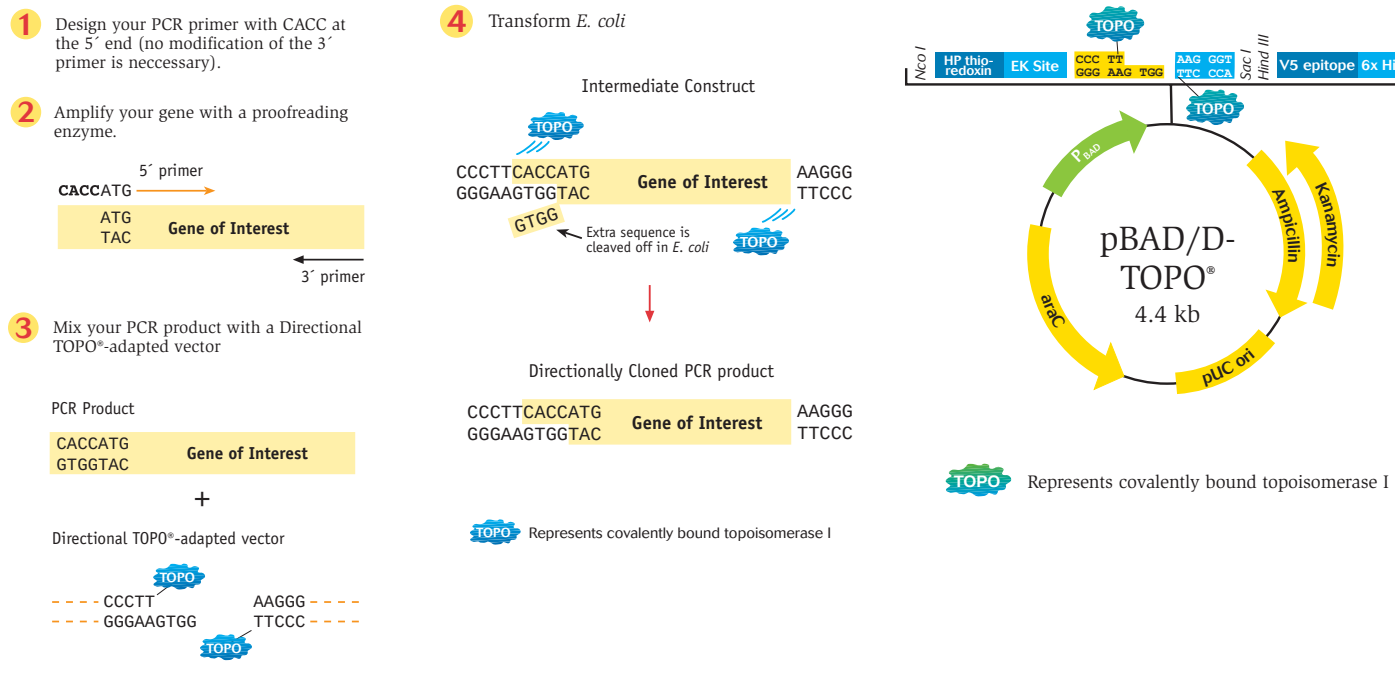
- *araBAD* promoter for dose-dependent regulation
- *ara*C gene for tight control of the *araBAD* promoter
- Optimized ribosome binding site for increased translation efficiency
- *rrn*B transcription termination region for efficient transcript processing

Read on to find out more about these versatile vectors.

Directional TOPO® Cloning: 5 minute cloning, reduced screening

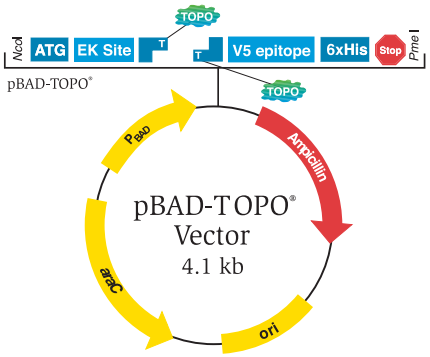
The pBAD Directional TOPO® Expression Kits offer efficient cloning of blunt-ended PCR products generated with proofreading DNA polymerases. A choice of ampicillin (pBAD102/D-TOPO®) or kanamycin (pBAD202/D-TOPO®) resistance markers give you the flexibility to design your experiments the way you want to. The mechanism for Directional TOPO® Cloning is simple (Figure 4). Once the PCR product and vector are mixed, a four base pair overhang on the TOPO®-adapted vector hybridizes to the complimentary sequence in your PCR product via strand invasion. The topoisomerase I then rapidly performs a ligation that results in greater than 90% of recombinant clones in the correct orientation for expression. The entire reaction only takes five minutes and maintains directionality, so you spend less time screening.

Figure 4 - How Directional TOPO® works



TOPO® Cloning: 5 minute cloning

The pBAD TOPO® TA Expression Kit specifically is designed for one-step cloning and regulated expression in *E. coli*. *Taq*-amplified PCR fragments are ligated in just 5 minutes and result in ≥95% recombinants. First clone your PCR product with TOPO®, then go straight to protein expression. The expression vector, pBAD-TOPO®, contains fusion tags to make protein detection and purification even easier.



TOPO Represents covalently bound topoisomerase I

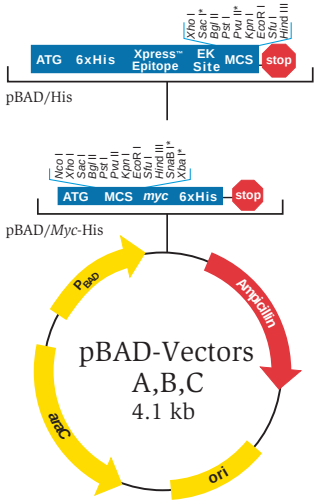
The pBAD TOPO® TA Expression Kit offers:

- Topoisomerized vector for 5-minute TOPO® Cloning of *Taq*-generated PCR products
- Enterokinase cleavage site to remove thioredoxin after protein purification (if desired)
- C-terminal V5 epitope for detection and analysis with the Anti-V5 antibodies
- C-terminal 6xHis tag for rapid purification with ProBond™ resin and detection with the Anti-His(C-term) antibodies

Choose your vector

Cytoplasmic expression

pBAD/His and pBAD/*Myc*-His encode an N- or C-terminal fusion tag, simplifying purification and detection of your protein of interest.



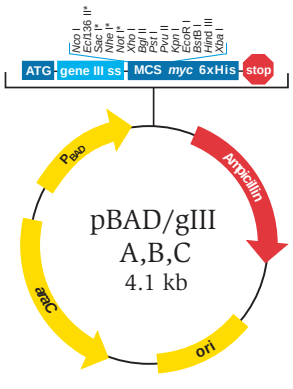
* Frame-dependent variations

- N-terminal polyhistidine (6xHis) Tag (pBAD/His) or C-terminal 6xHis Tag (pBAD/*Myc*-His) for rapid purification with ProBond™ resin and detection the an Anti-His(C- term) antibodies (pBAD/*Myc*-His only)
- N-terminal Xpress™ epitope for detection and analysis with an Anti-Xpress™ Antibody (pBAD/His only)
- Enterokinase cleavage site for removing the N-terminal tag following purification (pBAD/His only)
- C-terminal c-*myc* epitope for detection and analysis with the Anti-*myc* antibodies (pBAD/*Myc*-His only)

Periplasmic expression

pBAD/gIII allows secretion of the expressed protein into the periplasmic space, where oxidative conditions favor the formation of structural disulfide bonds for the production of functional proteins.

Periplasmic secretion also separates the recombinant protein from cytosolic proteases. The outer membrane can be easily disrupted, releasing the periplasmic proteins and thereby simplifying the purification of recombinant proteins.



* Frame-dependent variations

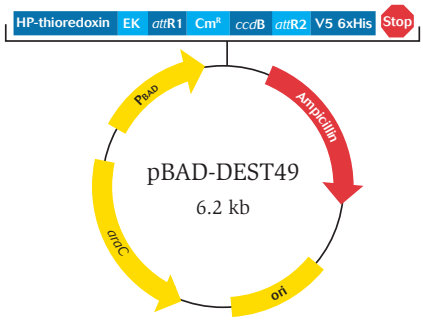
- Leader peptide from the bacteriophage fd gene III (gIII) protein for secreted expression into the periplasm
- C-terminal c-*myc* epitope for detection and analysis with the Anti-*myc* antibodies
- C-terminal 6xHis Tag for rapid purification with ProBond™ resin and detection with the Anti-His (C-term) antibodies

Quick and easy expression in multiple systems

Save time, accelerate discovery. The pBAD Expression System is part of the Gateway™ Technology, a powerful platform that enables you to quickly and easily clone genes and express them in any number of different expression systems. Gateway™ eliminates repetitive subcloning and sequencing steps while conserving reading frame and orientation.

Gateway™-adapted pBAD expression vector

Gateway™ Technology is based on the well-characterized system for site-specific recombination in phage lambda. To use Gateway™, first clone your gene of interest into an entry vector. Then recombine the entry construct with as many different Gateway™-adapted expression vectors (destination vectors) as you like. The end result is an expression clone that contains your gene of interest in the destination vector. The tightly regulated *araBAD* promoter of the pBAD-DEST49 vector enables you to express toxic and growth-inhibitive proteins in *E. coli* and offers:



- C-terminal V5-epitope for detection N-terminal thioredoxin fusion partner for improved translation effiency and increased protein solubility
- C-terminal 6xHis tag for rapid purification •
- Enterokinase cleavage site to remove thioredoxin after protein purification (if desired)
- *att*R sites for recombination with Gateway™ entry clone C-terminal V5 6xHis tag

For more information on Gateway™ Technology visit www.invitrogen.com.

Invitrogen™
life technologies

The ultimate expression advantage

Each pBAD kit comes complete for your convenience. pBAD102/D-TOPO®, pBAD202/D-TOPO®, pBAD-TOPO® and pBAD/Thio-TOPO® are linearized and topoisomerase-activated. pBAD-DEST49 is supplied as a stand-alone vector. pBAD/His, pBAD/*Myc*-His, and pBAD/gIII are supplied in three reading frames for easy cloning in-frame with the purification/detection tag. Each kit is provided with a positive control to help you troubleshoot your expression and purification process. Bacterial strains, 20% arabinose, and detailed instruction manuals are included with all kits. See how the pBAD Expression System can make a difference in your bacterial expression experiments. Call Invitrogen and order a kit today.

Product	Quantity	Cat. no.
pBAD102/Directional TOPO® Expression Kit	1 kit	K4102-01
pBAD202/Directional TOPO® Expression Kit	1 kit	K4202-01
pBAD/TOPO® Thiofusion™ Expression System	20 rxns	K370-01
pBAD TOPO® TA Expression Kit	20 rxns	K4300-01
	40 rxns	K4300-40
pBAD/His A, B, & C	1 kit	V430-01
pBAD/*myc*-His A, B, & C	1 kit	V440-01
pBAD/gIII A, B, & C	1 kit	V450-01
pBAD/Thio-E	1 kit	ET100-01
pBAD-DEST49 Vector	6 µg	12283-016
Additional Products		
Anti-myc Antibody +	50 µl	R950-25
Anti-Xpress™ Antibody‡	50 µl	R910-25
Anti-His(C-term) Antibody +	50 µl	R930-25
Anti-HisG Antibody#	50 µl	R940-25
Anti-V5 Antibody +	50 µl	R960-25
Positope™ Control Protein	5 µg	R900-50
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Resin	50 ml	R801-01
	150 ml	R801-15
EKMax™ Enterokinase	250 units	E180-01
	1000 units	E180-02
EK-Away™ Resin	7.5 ml*	R180-01
	30 ml*	R180-02

* 7.5 ml of resin will remove 250 units of EKMax™
30 ml of resin will remove 1000 units of EKMax™
+ FITC-, AP- and HRP- conjugated antibodies also available
#AP- and HRP-conjugated antibodies also available
‡HRP and FITC-conjugated antibodies also available

The pBAD Expression System has been licensed to Invitrogen Corporation and is sold for research purposes only.

References:

1. Guzman, L-M. *et al.* (1995) *J. Bacteriology* **177:** 4121-4130.
2. Smith, B. *et al.* (1978) *J. Bio. Chem.* **253:** 6931-6933.
3. Hirsh, J. *et al.* (1977) *Cell* **11:** 545-550.
4. Ogden, S. *et al.* (1980) *Proc. Natl. Acad. Sci. USA* **77:** 3346-3350.
5. Soisson, S.M. *et al.* (1997) *Science* **276:** 421-425.
6. Crameri, A. *et al.* (1996) *Nature Biotechnology* **14:** 315-319

Invitrogen™
life technologies

Corporate headquarters:
1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com
European headquarters:
Invitrogen Ltd, 3 Fountain Drive • Inchinnan Business Park • Paisley PA4 9RF, UK • Tel: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260 • E-mail: eurotech@invitrogen.com

710-01619 051002 xxM